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Distribution of Epstein-Barr Virus Antigenic Sites on the Carboxyl Terminal End of Ribonucleotide Reductase Against Nasopharyngeal Carcinoma Serum Antibodies Using an Immunoabsorption Method

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In an attempt to clone and express proteins from the Epstein-Barr virus (EBV) cDNA library to be used as antigens in an enzyme-linked immunosorbent assay (ELISA) format to test against the antibodies found in the sera of nasopharyngeal carcinoma (NPC) patients, we have isolated and characterized three clones. All three clones expressed the same polypeptides of different lengths, which belong to the carboxyl terminal end of the large subunit of ribonucleotide reductase (RR) of the EBV genome. All three clones were found to be immunogenic and could be used in an IgA and IgG ELISA against the NPC sera with various degrees of sensitivity and specificity. Because the clones varied in length, this difference provides a simple system to determine where most of the antibody epitopes lies on the protein. We designed an immunoabsorption assay and a mathematical model to help map the segment of the polypeptide most immunogenic to 43 NPC patients. Results were unexpected: 77% of the patients were most immunogenic to region **z**, which was the smallest fragment among the three fragments studied. Fragment **z** was only 33 amino acids in length. Only 14% and 19% of patients showed the most immunogenic region in segment **x** and **y**, respectively. This variation could be due to major histocompatibility complex antigens. The patients could be divided into three groups based on the immunoabsorption assays, in which each group responded to a different immunodominant segment in the RR antigen. The largest group responded to an immunodominant segment, which was only 33 amino acids long. This domain was coded for by the gene fragment from nucleotide 78,129 to nucleotide 78,227 of the EBV genome. This segment of the protein would be suitable for further epitope mapping studies. ***J. Med. Virol. 58:385–396, 1999.***

KEY WORDS: immunoabsorption; ELISA; Epstein-Barr virus; nasopharyngeal carcinoma; epitope mapping

INTRODUCTION

Epstein-Barr virus (EBV) is believed to be associated with the etiology of nasopharyngeal carcinoma (NPC). IgA antibodies toward the viral capsid antigen (VCA) and early antigen (EA) had been correlated with NPC. Therefore, the detection of these antibodies in the serum of individuals using indirect immunofluorescence assays (IFA) has been the conventional test to diagnose NPC [Henle et al., 1970; Henle and Henle, 1976]. With the advent of monoclonal antibody technology and gene cloning, it is now possible to identify and purify different EBV polypeptides for use in enzyme-linked immunosorbent assays (ELISA) for the diagnosis of NPC [reviewed by Gan et al., 1996]. This approach has greatly increased the sensitivity and specificity of ELISA and represents a suitable test for mass screening of populations at high risk for NPC because it is easily automated, quick, and easy to perform. Employing gene

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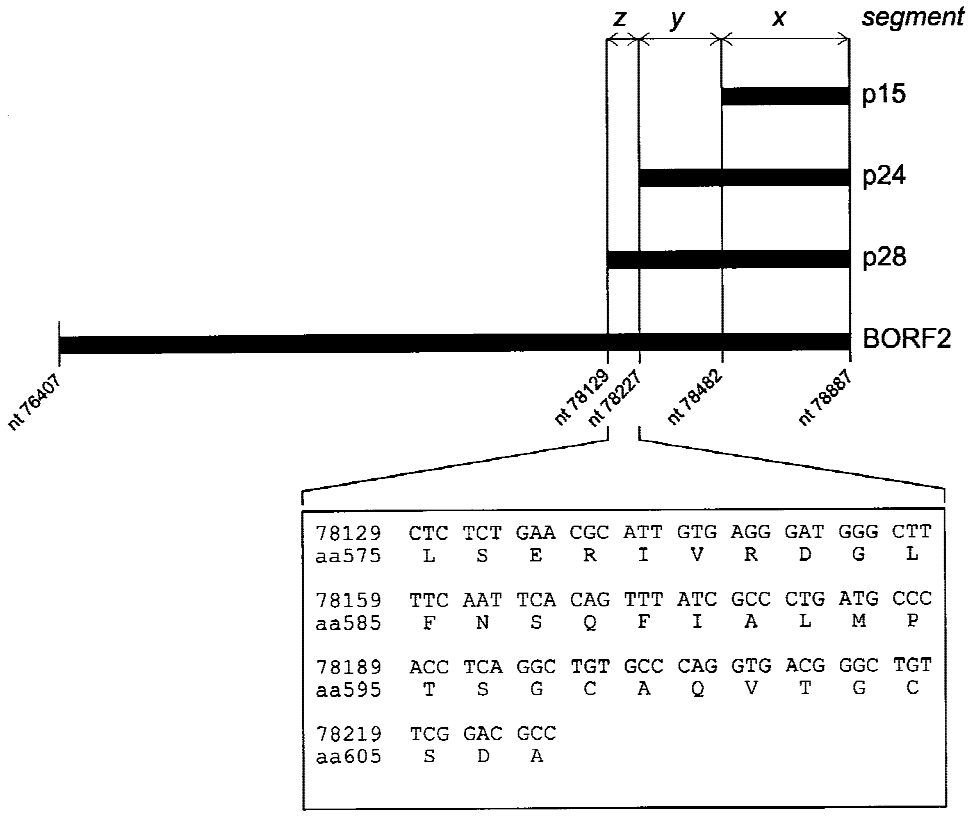

Fig. 1. The three cDNA clones corresponding to the three antigens, p15, p24, and p28, used in this immunoabsorption study compared with the Epstein-Barr virus (EBV) BORF2 open reading frame, which codes for the large subunit (gp85) of ribonucleotide reductase. The nucleotide and amino acid sequence for segment **z** is shown. Nucleotide (nt) and amino acid (aa) numbering reflects the nucleotide position in the complete genome sequence and amino acid position in gp85, respectively.

cloning and expression, several groups had used various recombinant EBV polypeptides to evaluate their reactivity with the antibodies from NPC patients with varying success. Such studies included polypeptides of the VCA complex [Uen et al., 1988], the membrane antigen (MA) complex [Uen et al., 1988; Littler et al., 1991; Yao et al., 1991; Durda et al., 1993], the EA complex [Liu et al., 1989; Ginsburg, 1990; Baylis et al., 1991; Chen et al., 1991; Joab et al., 1991; Littler et al., 1991; Chen et al., 1993; Gan et al., 1994; Matthew et al., 1994; Liu et al., 1997], the EBV nuclear antigen (EBNA) complex [Foong et al., 1990], and others [reviewed by Gan et al., 1996].

Similarly, it was shown that a polypeptide from the carboxyl end of the large subunit of ribonucleotide reductase (RR), p24, was immunogenic and could be used in an IgA and IgG ELISA to elicit 60% and 81% sensitivity and 91% and 99% specificity, respectively, for the diagnosis of NPC [Fones-Tan et al., 1994]. Ribonucleotide reductase is composed of a large and a small subunit and has been identified as a component of the restricted early antigen complex (EA-R). The large subunit is a 85-kDa glycoprotein (gp85), coded by the BORF2 reading frame at nucleotides 76,407 to 78,887 of the EBV genome (strain B95-8) [Baer et al., 1984]. In addition, we had generated another two polypeptides, p15 and p28, both of which belong to the carboxyl terminus of the RR antigen. Since p15, p24, and p28 (Fig. 1) originated from the same carboxyl terminus but differed in length, this provided a simple system to determine where most of the antibody epitopes lies on the protein. An immunoabsorption assay was designed to help map the segment of the polypeptide most immunogenic to 43 NPC patients. The distribution of immunogenic epitopes would help in the design of the recombinant antigen. The sensitivity and specificity of the three polypeptides were also examined for the diagnosis of NPC in the IgG- and IgA-ELISA.

MATERIALS AND METHODS

Human Sera

NPC sera from IFA-VCA-IgA and IFA-EA-IgA positive histologically confirmed NPC cases were collected from the Cancer Center, Gleneagles Hospital and the WHO Immunology Center, National University of Singapore. Normal sera were obtained from the Blood Bank, National University Hospital, Singapore.

Construction of Recombinant Plasmids

The pMAL-c2 plasmid vector (New England Biolabs, Beverly, MA) was used as it allowed the cloned gene to be fused to the *malE* gene of *Escherichia coli,* hence expressing a maltose-binding protein (MBP) fusion.

This vector uses the strong, inducible P_{tac} promoter to give high-level expression of the cloned gene, whereas MBP is poorly immunogenic and does not interfere with the assay. Various open reading frames (ORFs) were polymerase chain reaction (PCR)-amplified from an EBV cDNA library created from the Raji cell line. Details of the construction and screening of this cDNA library have been reported previously [Gan et al., 1994]. The cDNA positive clones were amplified and restriction-digested with *EcoR* I and cloned into the *EcoR* I site of the pMAL expression vector. *E. coli* TB1 cells were transformed by the recombinant vectors and propagated as pure clones.

Expression and Purification of Recombinant Antigens

The recombinant clones were grown overnight at 37°C in LB broth with glucose and ampicillin. When the cell density reached 2×10^8 cells/ml, isopropylthiogalactoside (IPTG) (Promega, Madison, WI) was added to a final concentration of 0.3 mM and the culture was grown for additional 2 hr. All subsequent steps were carried out at 4°C or on ice. The cells were harvested by low speed centrifugation at 3,000 × *g* for 20 min, were resuspended in 1/20 vol of column buffer (10 mM Tris-HCl, pH 7.4; 200 mM NaCl; 1 mM ethylenediamine tetraacetic acid [EDTA]) and lysed by sonication. Cellular debris was pelleted by centrifugation at 10,000 × *g* for 30 min. The supernatant was collected as crude extract which was diluted 1:5 with column buffer and purified by cross-linked amylose-affinity chromatography. The MBP-fusion protein bound to the column was eluted with 10 mM maltose in column buffer.

ELISA

Both IgG and IgA assays were optimised for specificity (defined as the percentage negative results among normal control sera) for the diagnostic differentiation of NPC-positive and normal individuals with a slight concomitant loss in sensitivity (defined as the percentage of positive results among NPC-positive sera). Briefly, serum samples (NPC positive and negative) were diluted serially and tested on ELISA. Anti-human IgA or IgG horseradish peroxidase conjugate was also diluted serially and used as the secondary antibody in the ELISA. The optimal combination of serum and secondary antibody dilutions for IgA and IgG assays was selected to discriminate between NPC-positive and NPC-negative serum (Fig. 2). Serum samples diluted in 1:100 in dilution buffer in IgA assay and 1:20 in IgG assay were found to be most suitable, as shown in Figure 2.

To prepare for the ELISA, 75 ng/well of purified recombinant proteins were coated onto ELISA plates and incubated at 4°C for 22 hr. The plates were then blocked with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 3.5 hr at 37°C. After blocking, the plates were washed once by washing

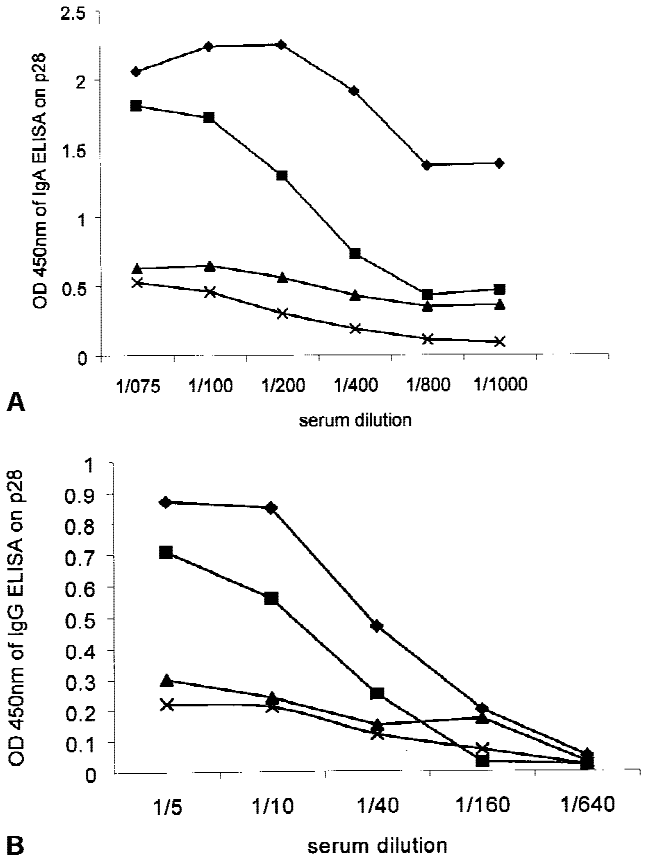

Fig. 2. Determination of the optimal serum dilution for the enzyme-linked immunosorbent assay (ELISA) of **(A)** IgA and **(B)** IgG when ribonucleotide reductase was used as antigens. Positive controls NPC1 (♦), NPC2 (■), and negative controls N1 (▲) and N2 (**X**) were used.

TABLE I. Sensitivity and Specificity of Recombinant Ribonucleotide Reductase Antigens in ELISA

Ribonucleotide reductase antigens	Sensitivity (% positive among NPC patients)		Specificity (% negative among normal individuals)	
	IgG assay	IgA assay	IgG assay	IgA assay
p15	69	53	98	96
p24	81	60	99	91
p28	80	65	98	96

ELISA, ezyme-linked immunosorbent assay; NPC, nasopharyngeal carcinoma.
Patient sera were tested for IgG and IgA antibodies against the RR antigens using ELISA.

buffer (0.05% Tween-20 in PBS) and dried at 37°C. Serum samples were diluted 1:100 in dilution buffer (0.2% BSA in PBS) in IgA assay and 1:20 in IgG assay. After 1 hr of incubation at 37°C, the plates were washed five times in washing buffer. For the IgA assay, sheep anti-human IgA horse radish peroxidase conjugate (Silenus, Hawthorn, Victoria, Australia) diluted 1:4,000 in dilution buffer was added to the wells and incubated for 1 hr at 37°C. For the IgG assay, sheep

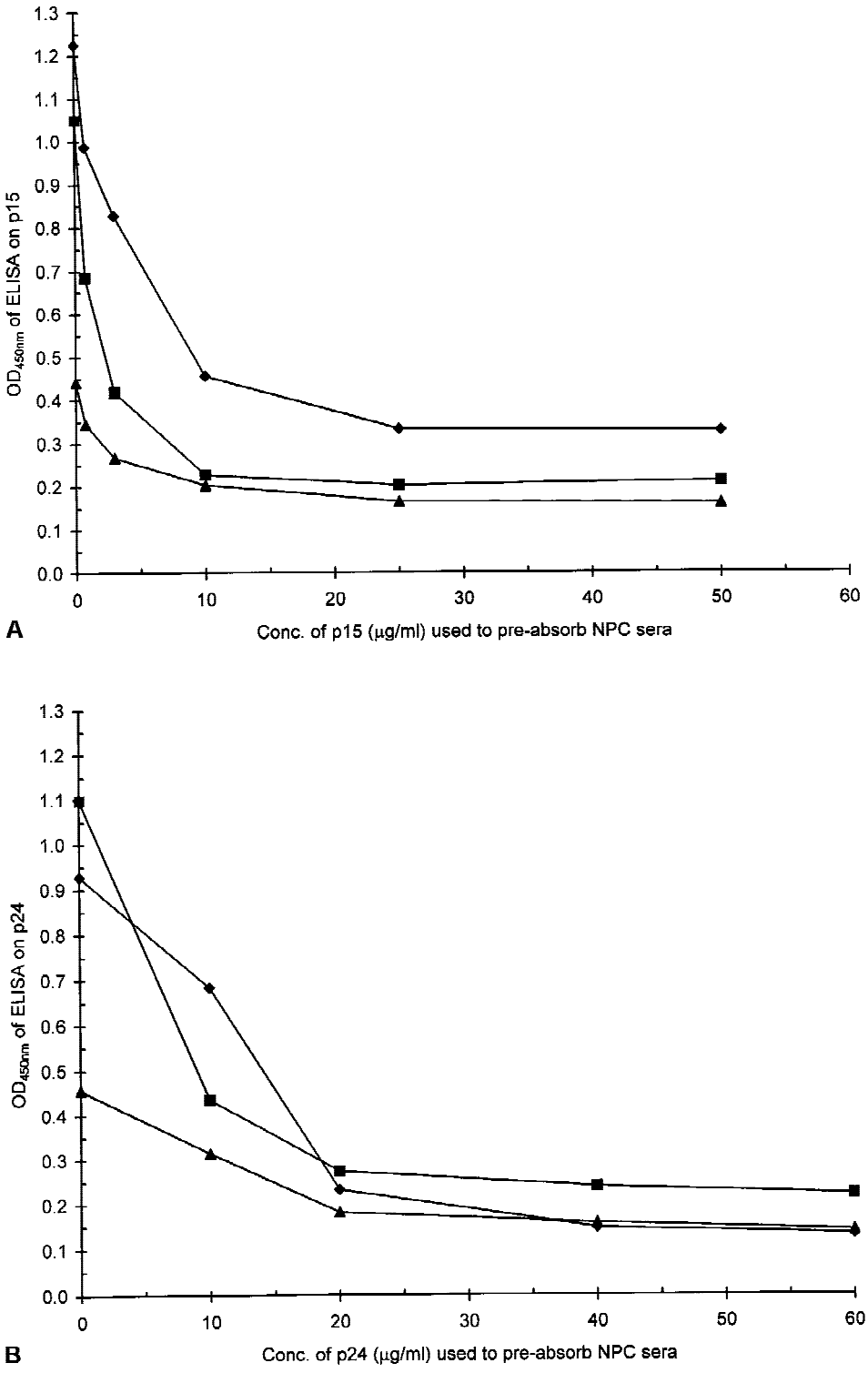

Fig. 3. Determination of the maximal amount of antigens **(A)** p15, and **(B)** p24 required to absorb the nasopharyngeal carcinoma (NPC) serum antibodies to saturation. Representative NPC serum samples NPC1 (♦), NPC4 (■), and NPC6 (▲) were used.

anti-human IgG horseradish peroxidase conjugate (Silenus) diluted 1:50,000 in dilution buffer was added instead. After incubation, the plates were washed five times in washing buffer. Finally, bound horseradish peroxidase conjugate antibody was detected by the enzymatic reaction with tetramethyl benzidine (TMB) (Sigma). After 30 min of incubation in the dark, the reaction was stopped by adding 2 N H_2SO_4 and absorbance at 450 nm was measured with a spectrophotometer.

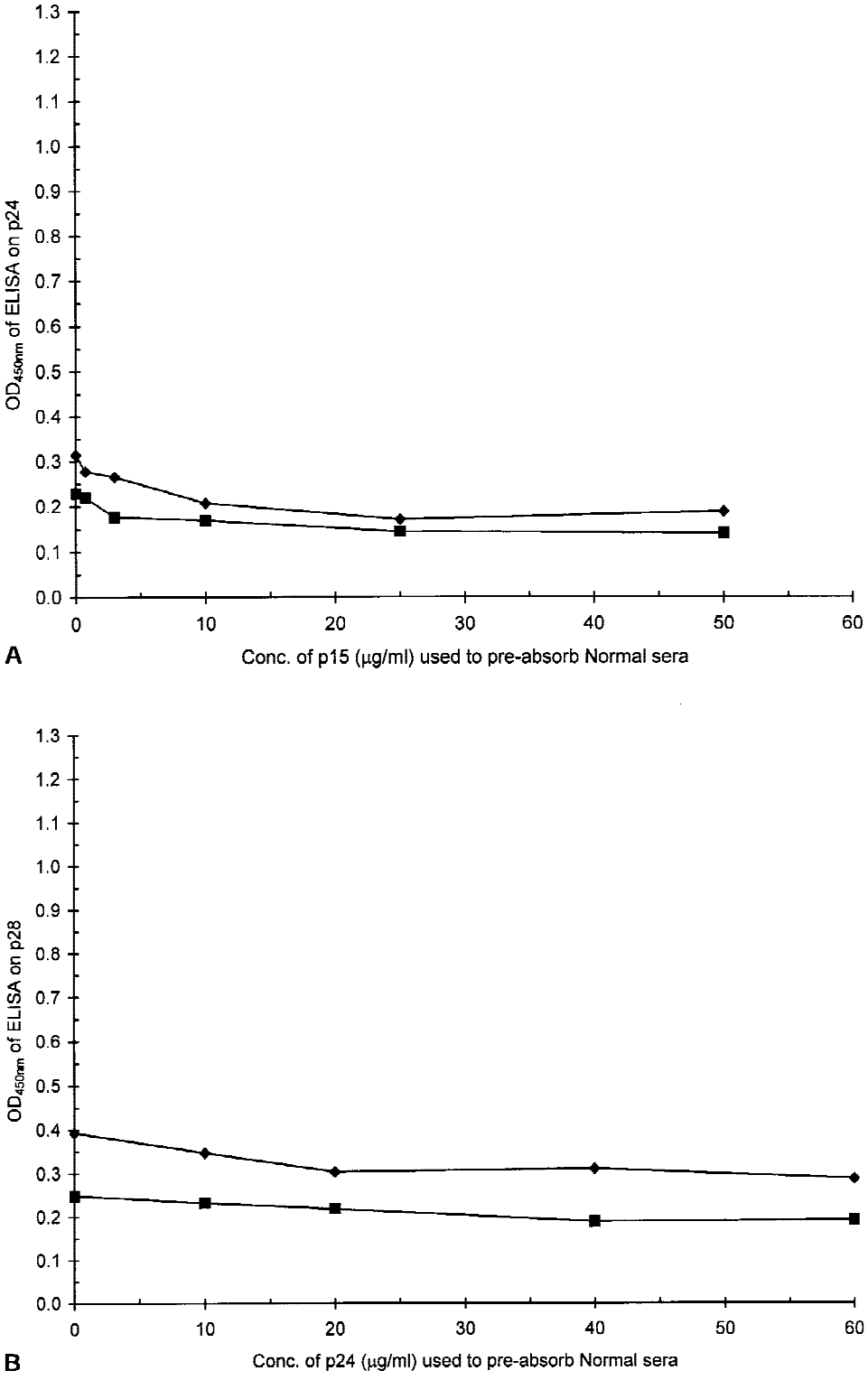

Fig. 4. OD readings of the normal serum samples, N1 (♦) and N2 (■), **(A)** preabsorbed with p15 and tested on enzyme-linked immunosorbent assay (ELISA) coated with p24 and **(B)** preabsorbed with p24 and tested on ELISA coated with p28. There was no significant decrease in OD for the two normal samples, indicating no serum antibodies against the antigenic sites on p15 and p24 were present.

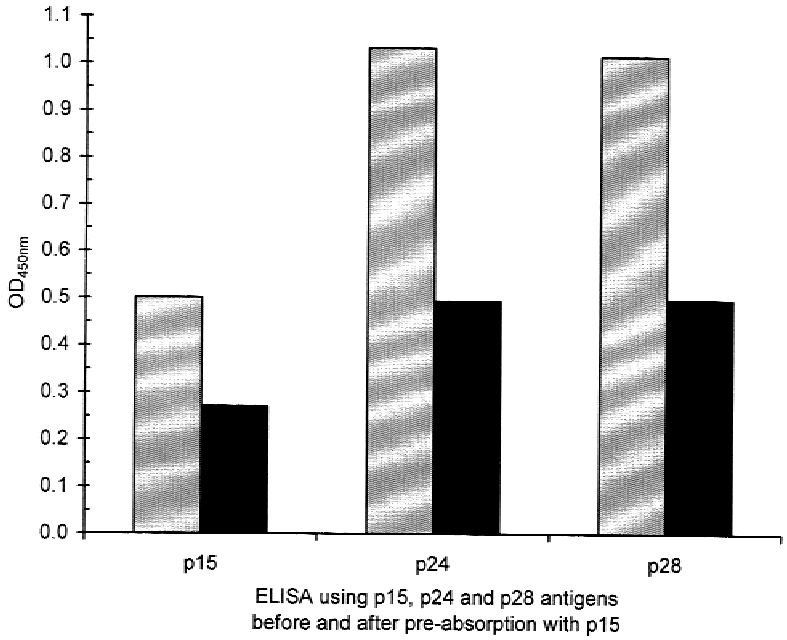

Fig. 5. The chart represents the OD readings of serum sample NPC1 before (gray) and after (black) preabsorption with p15 antigen. The difference in the two column heights for the same antigen represents the amount preabsorbed by the antigens.

All optical absorbance (OD) readings reflect the difference between the OD of tested samples and NPC-negative control serum. Only sera with OD readings of > 0.1 were considered positive.

IFA

Slides coated with P3HR1 cells fixed in cold acetone were used for IFA-VCA tests and slides coated with Raji cells fixed in cold acetone were used for IFA-EA test. Aliquots (10 μl) of serially diluted sera (1:5, 1:10, 1:40, 1:60, and 1:640) were added to the individual wells of the EBV VCA and EA slides. The slides were incubated in a moist chamber for 30 min at room tmeperature, then rinsed in PBS, and washed in PBS by immersing for 3 × 5 min with gentle agitation. The slides were drained and the sides of the wells were wiped with tissue paper. Fluorescein-conjugated anti-human IgA rabbit immunoglobulin (Dako, Glostrup, Denmark) (10 μl), diluted 60 × in PBS, was added to individual wells of the slides. The slides were then incubated in a moist chamber for 30 min at RT, rinsed, and washed as before. After draining off the PBS, mounting medium (2.25% DABCO, 90% glycerine, 10% PBS) was added and cover slips placed on. The slides were read under a fluorescence microscope at 40× objective (10× ocular). Titers of sera towards VCA and EA were taken as the highest serum dilution on that slide at which at least five brightly fluorescing cells could be seen over the whole well. Titers of 1:5 toward EA was considered positive. Titers of 1:10 toward VCA were taken as negative unless the sample was also EA positive, when it was also taken as VCA positive.

Immunoabsorption Assay

NPC-positive sera were mixed with increasing concentrations of p15 or p24, and then tested on ELISA plates coated with 5×10^{-8} M p15 or p24, respectively. The lowest concentration of the absorption antigen to result in a baseline OD reading was chosen as the working concentration of the absorption assay. The positive sera were mixed with the absorption antigens at a 1:100 dilution and incubated at room temperature for 1 hr. The mixture was then tested by ELISA.

RESULTS

Antibodies Against the Different RR Antigens

One hundred IFA-VCA- and IFA-EA-positive NPC patients were tested for IgA and IgG antibodies by ELISA by the recombinant RR antigen p24, while 156 such patients were similarly tested for p15 and p28. One hundred normal individuals were also tested for each antigen. In all the antigens tested, the IgG assay is more sensitive and specific for the detection of NPC (Table I). The range of sensitivity and specificity for both IgA and IgG assays in this study was comparable with previously reported ELISA-based studies when EBV antigens were used in the diagnosis of NPC as reviewed by Gan et al. [1996]. However, to compare the results with the IFA-EA-IgA titers, we decided to proceed with the immunoabsorption experiments using the IgA assay for mapping the immunodominant segment of the antigen. Forty-three NPC patient sera with OD readings > 0.1 for all three antigens were used for this immunoabsorption study.

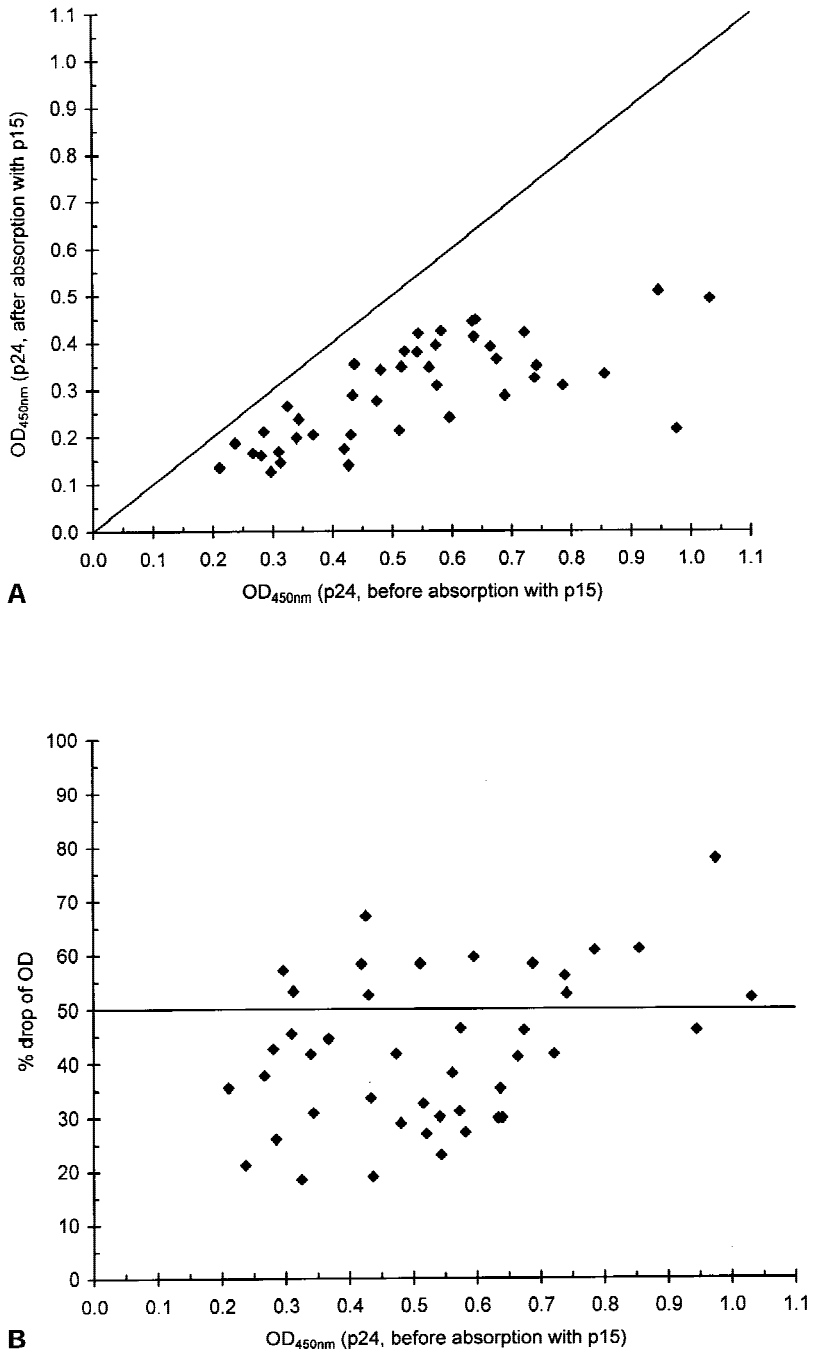

Fig. 6. **A:** The NPC samples were first absorbed by p15 and then tested on enzyme-linked immunosorbent assay (ELISA) coated with p24. The *X* and *Y* axes represent the ODs of the 45 nasopharyngeal carcinoma (NPC) samples before and after the absorption, respectively. **B:** Percentage of drop in ODs for the 45 NPC samples tested. The NPC samples were first absorbed by p15 and then tested on ELISA coated with p24.

Immunoabsorption of NPC-Positive Sera

To determine the concentrations of p15 and p24 antigens required for absorption, NPC-positive sera were mixed with increasing concentration of the relevant antigens as described in Materials and Methods. The concentration that yielded the baseline OD reading was chosen for subsequent assays. These were 25 μg/ml for p15 and 40 μg/ml for p24 (Fig. 3).

Determination of the Percentage of Antibodies Bound Through Immunoabsorption

We first examined sera from normal control individuals for immunoabsorption. Figure 3 shows two representative normal samples absorbed by p15 and then tested on ELISA coated with p24 (Fig. 4A), and the same samples absorbed by p24 and tested on p28 (Fig. 4B). There was no decrease in OD for normal samples

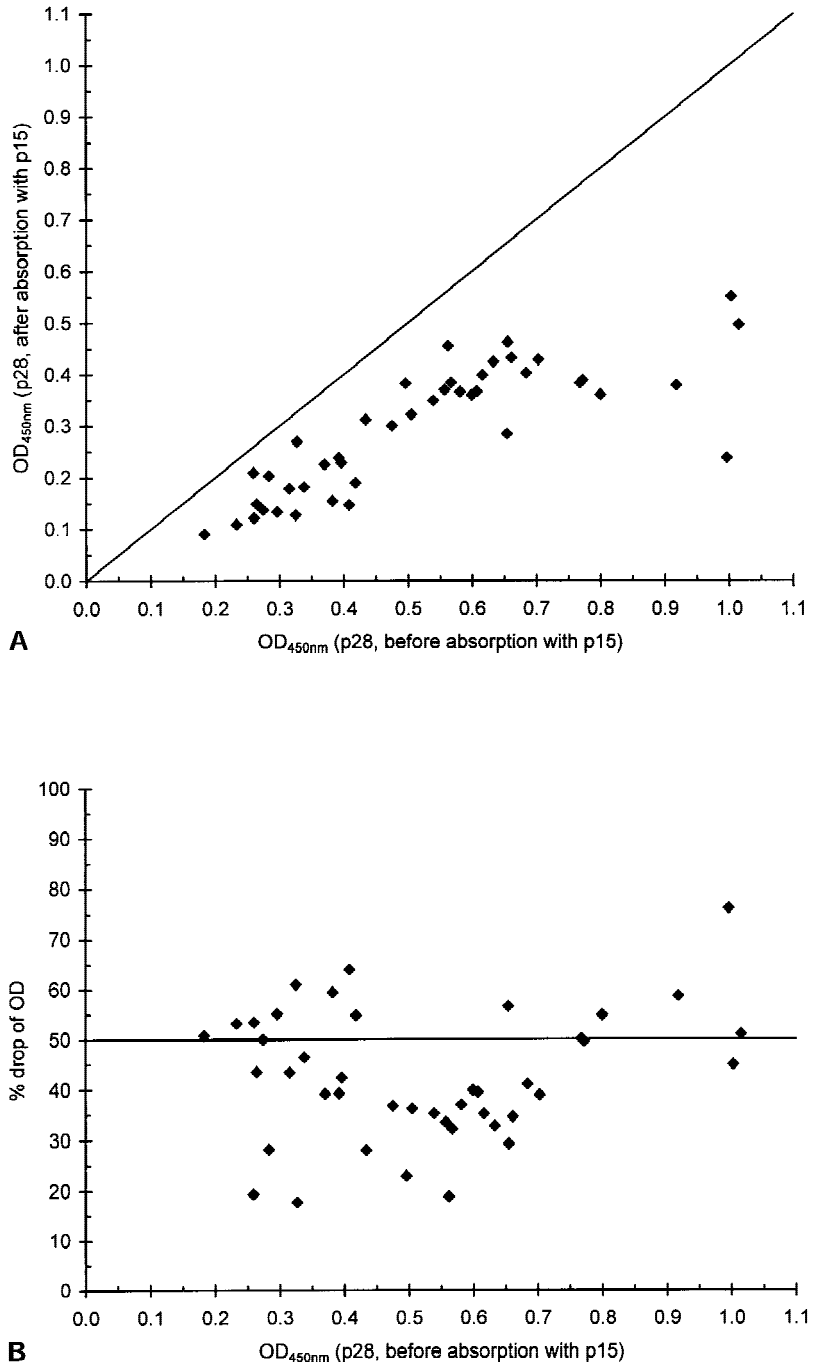

Fig. 7. **A:** The NPC samples were first absorbed by p15 and then tested on enzyme-linked immunosorbent assay (ELISA) coated with p28. The *X* and *Y* axes represent the ODs of the 45 nasopharyngeal carcinoma (NPC) samples before and after the absorption, respectively. **B:** Percentage of drop in ODs for the 45 NPC samples tested. The NPC samples were first absorbed by p15 and then tested on ELISA coated with p28.

after absorption, even with increasing concentration of antigens. The OD values for the normal sera before absorption were also low. This indicated that there were no serum antibodies in normal individuals against the antigens tested.

To determine the percentage of antibodies bound to different segments of the antigen, the sera were first absorbed on p15, and then tested by ELISA on p24 and p28. The difference in the OD values before and after absorption represents the amount preabsorbed by p15 (Fig. 5). This procedure was carried out similarly for the absorption by p24 and the subsequent test on p28. The data were then represented in scatter plots of OD after absorption against OD before absorption (Figs.

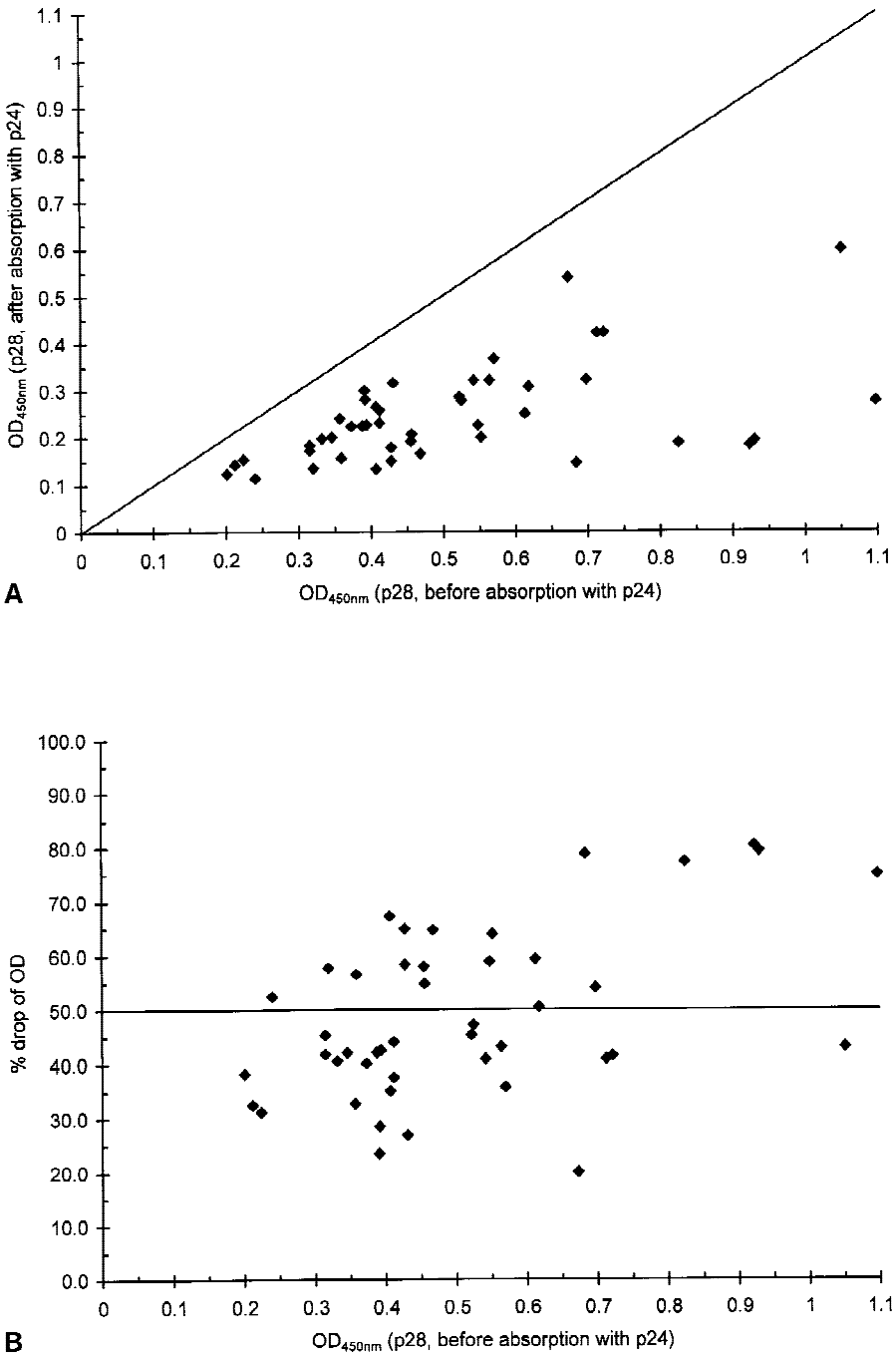

Fig. 8. **A:** The nasopharyngeal carcinoma (NPC) samples were first absorbed by p24 and then tested on enzyme-linked immunosorbent assay (ELISA) coated with p28. The *X* and *Y* axes represent the ODs of the 45 NPC samples before and after the absorption, respectively. **B:** Percentage of drop in ODs for the 45 NPC samples tested. The NPC samples were first absorbed by p24 and then tested on ELISA coated with p28.

6A, 7A, 8A), and the percentage decrease in OD after absorption against OD before absorption (Figs. 6B, 7B, 8B). Most patients showed significant absorption of their sera with p15 and p24, as evident by their readings, which fell below the line through the point of origin with a gradient of 1 (45° line) (Figs. 6A, 7A, and 8A). A sample data point on this line would indicate no change in OD before and after absorption. After absorption on p15, 14 of 43 (33%), and 16 of 43 patients (37%) had a 50% or greater decrease in their p24 and p28 OD readings, respectively. This finding showed that more patients had a majority of antibodies binding to epitopes outside of p15 (Figs. 6B, 7B). Nineteen of 43 patients (44%) had more than 50% antibodies binding

TABLE II. Mapping of Immunodominant Segments by the Relative Ratio of Antibodies Binding to Each Segment

Sample	IFA-EA-IgA Titer	Percentage of Antibodies Pre-absorbed / Bound to Test Antigen						Antigenicity				
		p24 ELISA pre-absorbed with p15		p28 ELISA pre-absorbed with p15		p28 ELISA pre-absorbed with p24		Relative Ratio of Bound Antigens by Segment, where (x+y+z) =1			Segments with the most bound antibodies	
		Absorbed by p15	Bound to p24	Absorbed by p15	Bound to p28	Absorbed by p24	Bound to p28					
		x	y	x	yz	xy	z	x	y	z	1st	2nd
1	1:160	52	48	51	49	79	21	**0.411**	0.379	0.210	x	y
3	1:40	61	39	59	41	77	23	**0.470**	0.300	0.230	x	y
22	-	58	42	35	65	80	20	**0.464**	0.336	0.200	x	y
36	1:40	58	42	53	47	79	21	**0.458**	0.332	0.210	x	y
4	-	84	16	76	24	75	25	**0.630**	0.120	0.250	x	z
39	< 1:5	56	44	55	45	67	33	**0.375**	0.295	0.330	x	z
18	-	38	62	35	65	65	35	0.247	**0.403**	0.350	y	z
20	< 1:5	35	65	33	67	64	36	0.224	**0.416**	0.360	y	z
32	1:40	27	73	40	60	59	41	0.159	**0.431**	0.410	y	z
40	≥ 1:640	46	54	45	55	65	35	0.299	**0.351**	0.350	y	z
5	1:10	57	43	59	41	55	45	0.314	0.237	**0.450**	z	x
6	1:5	53	47	50	50	42	58	0.223	0.197	**0.580**	z	x
13	1:40	60	40	57	43	43	57	0.258	0.172	**0.570**	z	x
21	< 1:5	58	42	39	61	57	43	0.331	0.239	**0.430**	z	x
23	-	67	33	46	54	45	55	0.302	0.149	**0.550**	z	x
30	-	53	47	55	45	40	60	0.212	0.188	**0.600**	z	x
37	1:40	61	39	50	50	59	41	0.360	0.230	**0.410**	z	x
41	1:5	53	47	50	50	58	42	0.307	0.273	**0.420**	z	x
2	< 1:5	19	81	36	64	38	62	0.072	0.308	**0.620**	z	y
7	< 1:5	42	58	39	61	58	42	0.244	0.336	**0.420**	z	y
8	1:40	41	59	41	59	41	59	0.168	0.242	**0.590**	z	y
9	< 1:5	30	70	35	65	27	73	0.081	0.189	**0.730**	z	y
10	1:10	30	70	34	66	33	67	0.099	0.231	**0.670**	z	y
11	1:10	19	81	40	60	24	76	0.046	0.194	**0.760**	z	y
12	< 1:5	31	69	42	58	45	55	0.140	0.311	**0.550**	z	y
14	< 1:5	23	77	19	81	20	80	0.046	0.154	**0.800**	z	y
15	1:40	21	79	19	81	31	69	0.065	0.245	**0.690**	z	y
16	1:40	42	58	64	36	41	59	0.172	0.238	**0.590**	z	y
17	1:40	27	73	23	77	43	57	0.116	0.314	**0.570**	z	y
19	1:10	31	69	32	68	58	42	0.180	0.400	**0.420**	z	y
24	≥ 1:640	26	74	44	56	35	65	0.091	0.259	**0.650**	z	y
25	1:40	34	66	39	61	36	64	0.122	0.238	**0.640**	z	y
26	1:5	43	57	53	47	38	62	0.163	0.217	**0.620**	z	y
27	1:160	42	58	61	39	44	56	0.185	0.255	**0.560**	z	y
28	1:160	36	64	51	49	33	67	0.119	0.211	**0.670**	z	y
29	< 1:5	45	55	55	45	47	53	0.212	0.259	**0.530**	z	y
31	< 1:5	30	70	29	71	42	58	0.126	0.294	**0.580**	z	y
33	< 1:5	45	55	43	57	53	47	0.239	0.292	**0.470**	z	y
34	1:40	33	67	18	82	42	58	0.139	0.281	**0.580**	z	y
35	1:40	46	54	28	72	51	49	0.235	0.275	**0.490**	z	y
38	1:160	29	71	37	63	42	58	0.122	0.298	**0.580**	z	y
42	1:160	46	54	37	63	41	59	0.189	0.221	**0.590**	z	y
43	< 1:5	38	62	28	72	29	71	0.110	0.180	**0.710**	z	y

NPC Patient sera were pre-absorbed with p15 or p24 and tested on ELISA plates coated with p24 or p28. Data represent the percentage of antibodies bound to each segment (*x,y,* or *z*) of the antigens. Ratio of bound antibodies per segment was calculated by taking the ratio of *x* & *y* (column 3 & 4) out of the percentage of antibodies bound to p24 (*xy;* column 7) to give the antibodies bound to *x, y,* and *z* (column 8). Samples were then ranked according to immunodominant segment.

to p24 than the region outside it (Fig. 8B). This number was only slightly higher than the absorption data for p15 (37%), indicating that many people had more than 50% antibodies against epitopes beyond p24 and within p28.

A more quantitative representation of the percentage of antibodies bound to each segment is shown in Table II. The segment corresponding to the full length of p15 (**x**) is most immunogenic for 6 patients (14%), whereas the amino terminal segment of p24 (**y**) and amino terminal segment of p28 (**z**) are most immunogenic for 4 (19%) and 33 (77%) patients, respectively. Therefore, most patients produced most antibodies against the epitopes in the **z** segment of the carboxyl end of the RR large subunit. However, we did not find any correlation between the IFA titers and the distribution of immunodominant epitopes on any segment (Table II).

The DNA sequence of segment **z** from the cDNA clone coding for p28 matched the known sequence of the BORF2 open reading frame (B95-8 strain). The 98 nucleotide sequence codes for 33 amino acids, the 33rd codon overlaps segment **y** (Fig. 1).

DISCUSSION

In this study, an immunoabsorption assay was designed to estimate the distribution of antibody epitopes in the carboxyl terminus of the RR protein. The carboxyl end of the protein has been shown to be immunogenic, with high sensitivity as well as specificity when used as antigens in ELISA for the diagnosis of NPC patients (Table I). Therefore, the immunoabsorption assay would allow us to determine if there were an immunodominant segment and whether this would vary among the patients.

Among the six patients showing the most immunodominant region in **x**, four of them (9%) had **y** as the second most immunodominant region, and two of them (4.5%) had **z** as the second most immunodominant region (refer to Table II and Fig. 1). All four patients showing **y** as the most immunodominant region had **z** as the second most dominant region.

Among the 33 patients showing **z** as the most immunodominant region, 8 of them (24%) had **x** as the second immunodominant region and 25 of them (76%) had **y** as the second most immunodominant region.

The absorption data showed that most patients had more IgA antibodies against epitopes beyond p15, which is in agreement with the increased sensitivity of the IgA ELISA using p24 and p28 compared with p15 (Table I). This indicates the necessity of using the longer antigen for detection in NPC diagnosis. It was initially surprising to see that 33 of the 43 patients (77%) had most antibodies mapping to the segment **z** because this region was the shortest among the three, only 33 amino acids in length. However, it is possible that many of these antibodies recognized epitopes formed by residues spanning the boundary between segment **y** and segment **z**. The antibodies could be recognizing both linear epitopes and conformation-dependent epitopes that spanned segments **x, y**, and **z**. As a result, these antibodies would not be absorbed by p24 alone and would be detected as those binding to segment **z**.

The patients could be divided into three groups based on the immunoabsorption assays, in which each group responded to a different immunodominant segment in the RR antigen. The largest group responded to an immunodominant segment that was only 33 amino acids long; this domain was coded for by the gene fragment from nucleotide 78,129 to nucleotide 78,227 of the EBV genome. This segment of the protein would be suitable for further epitope mapping studies. No significant correlation was found between or within the groups to the IFA titer or to the ELISA OD reading, sensitivity, or specificity. The varied humoral response to the RR large subunit antigen could be partly due to genetic differences in major histocompatibility complex (MHC)-mediated antigen processing.

This immunoabsorption study was undertaken to determine the immunodominant segment on the carboxyl terminal end of the RR large subunit protein and whether it might vary among NPC-positive patients. The results would be useful in highlighting segments of the antigen for more precise epitope mapping. Identifying these epitopes could prove important in EBV vaccine design and have good potential in diagnostic applications using synthetic peptides. In addition, this method can be used to quantify the relative antigenicity of several overlapping polypeptides and thus can be used to select between clones of gene fragments from the same open reading frame generated by a cDNA library.

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